

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (Original) A DNA sequence coding for hG-CSF characterized in that the sequence comprises the nucleotide sequence of SEQ ID: 1.

Claim 2. (Currently amended) A DNA sequence characterized in that the sequence comprises a nucleotide sequence selected from the group ~~comprising~~: consisting of a combination of the following modifications with respect to the native hG-CSF sequence:

- in a "segment I" (located at the 5' terminal end between the nucleotide positions 3 and 194): a plurality of replacements which include replacements of *E. coli* rare codons by *E. coli* preference codons and replacements of GC rich regions by AT rich regions,
- in a "segment II" (located between the nucleotide positions 194 and 309): a plurality of replacements of *E. coli* rare codons by *E. coli* preference codons,
- in a "segment III" (located between the nucleotide positions 309 and 467): no change or essentially no change,
- in a "segment IV" (located at the 3' terminal end between the nucleotide positions 467 and 536): a plurality of replacements of *E. coli* rare codons by *E. coli* preference codons.

Claim 3. (Original) The DNA sequence according to claim 2, which encodes for a biologically active G-CSF.

Claim 4. (Currently amended) The DNA sequence according to ~~any one of~~ claims 1 to 3, wherein the nucleotide sequence is capable of providing an expression level of G-CSF, to the total proteins after expression, of at least 50%, ~~preferably at least 52%~~ in an expression system.

Claim 5. (Currently amended) The DNA sequence according to claim ~~4 or~~ 2, further comprising the 5'-untranslated region of the hG-CSF gene which are not changed relative to the native hG-CSF gene.

Claim 6. (Currently amended) An expression plasmid, ~~characterized in that~~ wherein the plasmid comprises the DNA sequence according to claim 1 ~~or 5~~ and a plasmid vector.

Claim 7. (Currently amended) An expression plasmid, ~~characterized in that~~ wherein the plasmid comprises a DNA sequence according to ~~any one of~~ claims 2 to 5 and a plasmid vector.

Claim 8. (Currently amended) An expression plasmid according to claim 6 ~~or 7~~, ~~characterized in that~~ wherein the plasmid vector comprises a T7 promoter sequence.

Claim 9. (Currently amended) An expression plasmid according to claim 6 ~~or 7~~, ~~characterized in that~~ wherein the plasmid vector is selected from the group of pET vectors.

Claim 10. (Currently amended) An expression plasmid according to ~~any one of claims 6 to 9~~, characterized in that the plasmid vector comprises a resistance gene, ~~preferably an~~ selected ~~from the group consisting of ampicilline or and a kanamycine resistance gene.~~

Claim 11. (Currently amended) An expression system for the expression of DNA sequence according to ~~claim 1~~ coding for hG-CSF characterized in that the sequence comprises the nucleotide sequence of SEQ ID: 1, ~~characterized in that~~ wherein the system comprises the expression plasmid according to ~~any one of claims 6 and 8 to 10~~ and a production strain *E. coli*.

Claim 12. (Canceled)

Claim 13. (Currently amended) The expression system according to claim 11 ~~or 12~~, characterized in that the production strain is *E. coli* BL21 (DE3).

Claim 14. (Currently amended) The expression system according to ~~any one of claims 11 to 13~~, characterized in that wherein it is used without an antibiotic.

Claim 15. (Currently amended) A process for construction of DNA sequence according to claim 1 ~~or claim 2~~, characterized in that wherein the process comprises

(i) applying methods in order to provide a DNA sequence which is changed relative to the native sequence coding for hG-CSF by:

- replacement of some *E. coli* rare codons with *E. coli* preference codons, and/or
- replacement of some GC rich regions with AT rich regions; and

(ii) maintaining a completely unchanged part in a substantial portion of the native sequence coding for hG-CSF.

Claim 16. (Original) A process for construction of DNA sequence according to claim 15, wherein the DNA sequence further comprises 5'-untranslated region of the hG-CSF gene, ~~characterized in that~~ wherein the process does not involve changes in the 5'-untranslated region in one or more of the following partial regions: translation initiation region, ribosome binding site and the region between the start codon and the ribosome binding site.

Claim 17. (Currently amended) The process for construction of DNA sequence according to claim 15 ~~or 16~~, wherein a completely unchanged sequence according to (ii) is maintained in segment III in a sequence of at least 99 nucleotides in length.

Claim 18. (Currently amended) The process for construction of DNA sequence according to ~~any one of claims 15 to 17~~, further comprising inserting said constructed DNA sequence into a plasmid vector which comprises a T7 promoter sequence.

Claim 19. (Currently amended) The process for construction of DNA sequence according to ~~any one of claims 15 to 18~~, which constructed DNA sequence is capable of providing an

expression level, to the total proteins after expression, of at least 50%, ~~preferably at least 52%~~ in a suitable expression system.

Claim 20. (Currently amended) A process for the expression of hG-CSF, comprising expressing the DNA sequence according to ~~any one of claims 1 to 5~~, or the expression plasmid according to ~~any one of claims 6 to 10~~ in *E. coli*.

Claim 21. (Original) The process for the expression of hG-CSF according to claim 20, wherein IPTG is used for induction at a concentration in the range of at least 0.1 mM to less than 1 mM, ~~preferably at a concentration of about 0.3 to 0.6 mM~~.

Claim 22. (Currently amended) The process according to claim 20 ~~or 21~~, which comprises a fermentation step that is performed at a temperature of about 20°C to 30°C, ~~preferably at around 25°C~~.

Claim 23. (Canceled)

Claim 24. (Currently amended) A process for the manufacture of a pharmaceutical composition ~~containing, as an effective ingredient, comprising~~ hG-CSF or biologically active G-CSF, ~~comprising steps of: wherein said process comprises:~~

- (a) carrying out a process according to ~~any one of claims 20 to 23~~,
- (b) isolating and/or purifying the hG-CSF or biologically active G-CSF obtained by step (a), and
- (c) mixing the isolated and/or purified hG-CSF or biologically active G-CSF with a pharmaceutically acceptable carrier or auxiliary substance.